Supramolecular Interaction between Adenosine 5′-Triphosphate (ATP) and Polycharged Tetraazamacrocycles. Thermodynamic and ^{31}P NMR Studies

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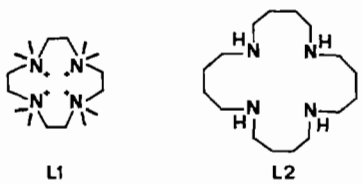
Abstract

The interaction of adenosine 5′-triphosphate (ATP) with the tetraammonium macrocyclic receptors 1,1,4,4,7,7,10,10-octamethyl-1,4,7,10-tetraazacyclododecane tetrakis(iodide) (L1·4I) and 1,6,11,16-tetraazacycloeicosane (L2) in its fully protonated form, has been studied by potentiometry and ^{31}P NMR in water at $I = 0.15$ mol dm^{-3} and 25 °C. H_4L2^{4+} reacts both with ATP^{4-} and $HATP^{3-}$ to produce $H_4L2 \cdot ATP$ and $(H_4L2 \cdot HATP)^{+}$ whose equilibrium constants are 6.46×10^3 and 1.10×10^3, respectively. In the case of L1, in which the quaternarization of the nitrogen atoms prevents the formation of hydrogen bonds, no detectable interactions arise with ATP. These results are consistent with the hypothesis that the formation of hydrogen bonds play a role of major importance in the interaction between ATP and tetrammonium receptors.

Introduction

Complexation of substrate molecules gives rise to substantial changes in the chemical reactivity and physicochemical properties of the substrate itself. Recently it has been shown that polyammonium macrocyclic receptors affect the electrochemical [1–4] and photochemical [5] behavior of the hexacyanide complexes of Fe(II), Ru(II) and Co(III), as well as the chemical behavior of some nucleotides [6, 7]. For instance, the rate of hydrolysis of ATP can be enhanced by complexation with polyazacycloalkanes in their protonated forms [6]. Naturally occurring receptors like spermine and spermidine have been studied for their *'in vivo'* interaction with ATP and hydrolysis promotion of its phosphate groups [8]. Synthetic macrocyclic polyamines offer the opportunity to gain an insight into the nature of these vital processes. The versatility of the synthetic procedures to obtain polyazamacrocycles with various sizes and structures and the ability of these macrocyclic compounds to bind many protons in the neutral pH region make polyazacycloalkanes powerful nucleotide receptors. Furthermore, the molecular architecture of the receptors can be planned in order to promote particular applications: for instance, large polyazapolyoxa- and polyazacycloalkanes have been designed for catalytic activation of ATP hydrolysis [6]; lipophilic diammonium salts for organic phase extraction and liquid membrane transport of nucleotides [9]. In this paper we report the results of a study of the interaction of ATP with 1,1,4,4,7,7,10,10-octamethyl-1,4,7,10-tetraazacyclododecane tetrakis(iodide) (L1·4I) and the fully protonated form of 1,6,11,16-tetraazacycloeicosane (L2). The two tetra-charged receptors have the same mass and charge but different sizes. Furthermore, in the case of L1, the ability to form hydrogen bonds with ATP is prevented by the full quaternarization of the nitrogen atoms.



Experimental

The synthesis of the tetraammonium macrocyclic receptors L1 and L2, together with the basicity constants of L2, have been reported elsewhere [4]. The disodium salt of adenosine 5′-triphosphate was purchased from Aldrich and used without further purification. $NaClO_4$, NaCl, HCl and CO_2-free NaOH solutions were obtained appropriately pure and standardized as already reported [10]. The interaction between ATP and L1 or L2 has been studied by potentiometric titration of solutions containing the nucleotide and one of the two receptors, in a 1:1 ratio, at 25.0 °C and 0.15 mol dm^{-3} aqueous $NaClO_4$ solution in the case of L2, or in 0.15 mol dm^{-3} NaCl in the case of L1 (perchlorate salts of L1 are water insoluble), by using the equipment already fully

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described [11]. The titrant was NaOH solution. The computer program SUPERQUAD [12] was used to process the potentiometric data and calculate the stability constants. Proton-decoupled ^{31}P NMR spectra were recorded at 32.2 MHz on a Varian CFT 20 spectrometer; the chemical shifts were obtained from 85% H_3PO_4 as external standard. The solutions used in the ^{31}P NMR measurements were 0.05 mol dm^{-3} ATP and 0.06 mol dm^{-3} of the ligand in D_2O/H_2O (2:8). The ionic strength, referring to the total concentration of Na^+, was adjusted to 0.15 mol dm^{-3} by addition of appropriate amounts of $NaClO_4$. The apparent pH of these solutions was measured by using a micro glass-electrode coupled with a Radiometer PHM 84 digital pH–mV meter.

Results and Discussion

The anion–receptor interaction in solution can take place when some peculiarity like complementary structures and pH compatibility are matched simultaneously by both the counterparts. The formation of the ATP anions and of the polyammonium cations (except the tetra-substituted amino derivatives) is strongly pH-dependent, so that the choice of the receptor for the binding of the nucleotide has to be carefully made. In the neutral pH region, the region in which these kinds of interactions obtain importance from a biological point of view, ATP is present as ATP^{4-} and $HATP^{3-}$ anions (Table I and Fig. 1). The overall basicity of L2 (Table I and Fig. 1) is high enough to allow formation of a large amount of the fully protonated species below pH 8.5, thus fulfilling the condition required for the recognition and binding of ATP anions. The species at equilibrium are rapidly formed and are stable with respect to hydrolysis of the phosphate groups, as shown by the ^{31}P NMR experiments. The logarithm of the overall stability constants, potentiometrically determined, for the reaction of formation of the nucleotide/polyammonium complexes

$$L2 + ATP^{4-} + 4H^+ \rightleftarrows H_4L2ATP$$

$$L2 + ATP^{4-} + 5H^+ \rightleftarrows (H_5L2ATP)^+$$

are 42.78 and 48.25, respectively. These complex species contain four and five protons respectively, a number smaller than the number of protonation sites virtually available on the ATP–L2 adduct. In the case of $(H_5(L2ATP)^+$, at least one proton has to be located at a phosphate group of the nucleotide, and the deprotonation reaction $(H_5L2ATP)^+ \rightleftarrows H_4L2ATP + H^+$ can take place, in principle, either on the macrocycle or on ATP. A comparison between the basicity constants of L2 and ATP^{4-} (Table I) strongly suggests the location of this proton at the nucleotide side. Nevertheless, the formation of adducts produces new species in which the basicity of a group may change; so, an assignment of the protonation sites cannot be unambiguously made only on the basis of potentiometric measurements. The ^{31}P NMR spectrum of ATP is pH-dependent, as depicted in Fig. 1a. The large downfield shift of the γ-phosphate signal occurs with the formation of the ATP^{4-} species and indicates that the proton present in $HATP^{3-}$ is located at the terminal phosphate group. On addition of L2, the signal of the γ-phosphate resonance starts shifting at lower pH values (Fig. 1b) as the H_4L2ATP species is formed from $(H_5L2ATP)^+$. Furthermore, no significant changes are detectable in the ^{31}P NMR spectra of H_4L2ATP and $(H_5L2ATP)^+$ with respect to those of ATP^{4-} and $HATP^{3-}$. These results allowed us to conclude that the fully protonated tetraazamacrocycle L2 forms complexes both with ATP^{4-} and $HATP^{3-}$. Therefore the formation reactions of the species H_4L2ATP and $(H_5L2ATP)^+$ may be written in the form:

$$H_4L2^{4+} + ATP^{4-} \rightleftarrows H_4L2 \cdot ATP$$

$$H_4L2^{4+} + HATP^{3-} \rightleftarrows (H_4L2 \cdot HATP)^+$$

and the relevant equilibrium constants may be calculated. The values obtained# (Table II) show clearly that the strongest interaction occurs between the

TABLE I. Logarithms of the Constants for the Protonation Equilibria of ATP and L2 Obtained by Potentiometric Titration at 25.0 °C, I = 0.15 mol dm^{-3} $NaClO_4$

Reaction	Log K
$ATP^{4-} + H^+ \rightleftarrows HATP^{3-}$	6.24(2)[a, b]
$HATP^{3-} + H^+ \rightleftarrows H_2ATP^{2-}$	4.00(2)[b]
$H_2ATP^{2-} + H^+ \rightleftarrows H_3ATP^-$	1.77(3)[b]
$L2 + H^+ \rightleftarrows HL2^+$	11.65(3)[c]
$HL2^+ + H^+ \rightleftarrows H_2L2^{2+}$	10.60(3)[c]
$H_2L2^{2+} + H^+ \rightleftarrows H_3L2^{3+}$	8.34(3)[c]
$H_3L2^{3+} + H^+ \rightleftarrows H_4L2^{4+}$	8.38(3)[c]
$L2 + 4H^+ \rightleftarrows H_4L2^{4+}$	38.97[c]

[a]Values in parentheses are the standard deviations on the last significant figure. [b]This work; values are in good agreement with those reported in the literature [13]. [c]Values taken from ref. 4.

#The stability constants reported in Tables I and II are conditional constants for 25.0 °C and I = 0.15 mol dm^{-3} $NaClO_4$. Owing to complex formation between Na^+ and ATP, they are valid for solutions in which the Na^+ concentration is about 0.15 mol dm^{-3}. As the chemistry of nucleotides is related to physiological solutions in which the Na^+ concentration is about 0.15 mol dm^{-3}, we have preferred to present the uncorrected stability constants. However, easy corrections can be carried out by using the formula reported in ref. 13.

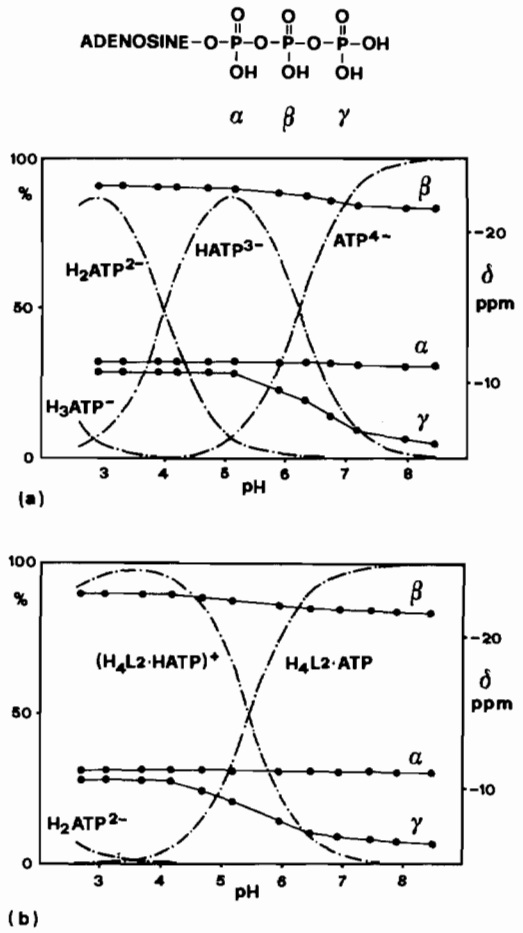

Fig. 1. Dependence on pH of the ^{31}P NMR chemical shifts of the α-, β-, and γ-phosphorus atoms of adenosine 5'-triphosphate in the absence (a) and in the presence (b) of 1,6,11,16-tetraazacycloeicosane (L2) in D_2O/H_2O (2:8). The distribution diagram of the species formed in the ^{31}P NMR experiments has been superimposed on the relative chemical shifts. Chemical shifts were obtained from 85% H_3PO_4 as external standard.

TABLE II. Logarithms of the Equilibrium Constants for the Complex Species Formed in the System ATP/L2 Obtained by Potentiometric Titration at 25.0 °C, $I = 0.15$ mol dm^{-3} $NaClO_4$

Reaction	Log *K*
$H_4L2^{4+} + ATP^{4-} \rightleftharpoons H_4L2{\cdot}ATP$	3.81(3)[a]
$H_4L2^{4+} + HATP^{3-} \rightleftharpoons (H_4L2{\cdot}HATP)^+$	3.04(4)

[a]Values in parentheses are the standard deviations on the last significant figure.

more charged species. The importance of the electrostatic contribution to the complexation of the anions has already been shown for some similar systems [1–4, 14, 15].

It has also been pointed out that other contributions, like hydrogen bond formation, increase the stability of such complexes. In order to improve our knowledge about the influence of hydrogen bonding in the chemistry of nucleotide anions, the interaction between ATP and L1 has been studied potentiometrically. In the case of L1, the formation of hydrogen bonds is prevented by quaternarization of the nitrogen atoms. Rather surprisingly we have found that no detectable interactions arise in solution between L1 and ATP. Four main contributions can be identified in determining the anion–cation interaction in solution: (i) a favourable entropic factor deriving from the large number of water molecules released as a consequence of the charge neutralization; (ii) an unfavourable enthalpic one arising from the desolvation itself; (iii) a favourable contribution, enthalpic in nature, due to the electrostatic attraction between two opposite charges; (iv) an enthalpic over-stabilization coming from the formation of hydrogen bonds between the two ions. In the case of L1 the fourth contribution cannot take place and the unfavourable factor (ii) overcomes the remaining favourable two. The completely different ability of the two geometrical isomers $L1^{4+}$ and H_4L2^{4+} to recognize and bind the adenosine 5'-triphosphate anions is clear evidence of the fact that the nature of supramolecular chemistry is not simply characterized by an ion-pair formation. The molecular architecture of the receptors from which a hydrogen bond network can arise toward the nucleotide anions is, in the present case, of major importance.

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